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DEVELOPMENT OF A PROCESS TO PURIFY HIGHLY VALUED INOSITOL FROM AN EXTRACT OF CANOLA

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Final Report on the ADF Project:

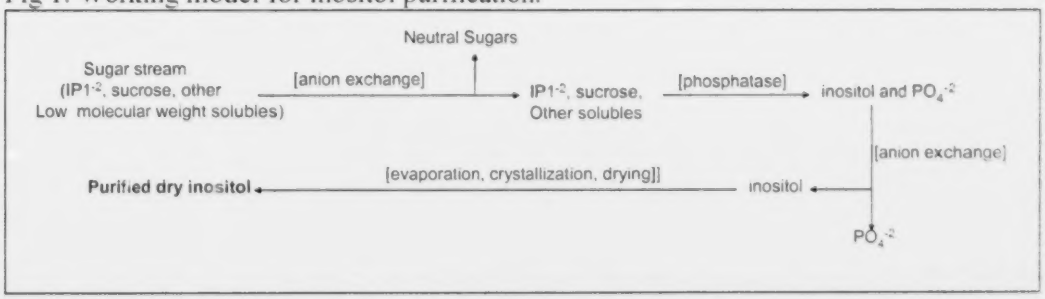
Development of a Process to Purify Highly Valued Inositol from an Extract of Canola

(March 5, 2004)

Background. MCN BioProducts Inc. is a growth stage technology development company working under a research contract with the University of Saskatchewan. MCN has developed a fractionation process to produce high valued canola protein concentrates from oil-extracted canola flakes. Phytic acid is an antinutritional factor present at high levels (3.2%) in conventional canola meal. Phytic acid is the fully phosphorylated form of inositol. Inositol is a high-valued B vitamin that is used for human consumption and as a feed additive to promote growth in shrimp production. Inositol is currently valued at $10,000 USD/mt. In the core process developed by MCN, the extract is treated with a commercial phytase to hydrolyze the phytic acid and thus reduce levels in the protein concentrate. Commercial phytase does not lead to 100% hydrolysis and the reaction stops at 2-mono-inositol phosphate (IP1). In the MCN process, IP1 accumulates in a stream of soluble low molecular weight sugars and minerals. IP1 contains a phosphate group and thus, at neutral pH, will exist as a negatively charged anion. Inositol is a neutral sugar similar in structure to glucose.

MCN developed a working hypothesis for the purification of inositol based on an initial separation of charged IP1 using an anion exchange resin, followed by complete hydrolysis to form neutral inositol. Inositol was then purified by anion exchange to remove charged compounds (fig 1). This theoretical process was the basis for the ADF value-added grant application.

Fig 1. Working model for inositol purification.



Results

Phase 1: Development of conditions for retention of IP1 from the sugar stream on a commercial anion exchange resin. Initial attempts at direct processing of the sugar stream through an anion exchange resin where largely unsuccessful. The sugar stream contained high levels of Cl that saturated the binding sites on the resin. Further, the sugar stream contained an unknown colored anionic compound that bound irreversibly to the resin. These factors precluded a simple protocol of direct removal of IP1 and other anionic compounds by passage through an anion exchange resin.

Removal of Cl from the sugar stream can be accomplished by desalination protocols. In the MCN core process, the sugar stream is generated by nanofiltration of the permeate from ultrafiltration. As such, the most cost effective method for desalination of the stream is to incorporate diafiltration (repeated rounds of water washing) during the nanofiltration step in the process. Low molecular weight components such as sodium and chlorine should readily pass through the nanofiltration membrane and thus should be "washed" from the sugar stream.

MCN conducted an assessment of component flows during diafiltration as added to the nanofiltration process. Interestingly, in addition to allowing flux of sodium and chlorine, the Osmonics DK nanofiltration membrane had poor rejection characteristics for glucose, fructose and inositol with considerable passage of these monosaccharides. The phosphorylated forms of inositol (including IP1) were retained by the membrane.

The combined finding of monosaccharide passage and inositol phosphate rejection by the nanofiltration membrane was unexpected. Apparently the phosphate group on the inositol molecule provides a combination of negative charge plus steric hindrance that serves to limit membrane passage relative to passage obtained with neutral monosaccharides such as inositol. As discussed below, this unexpected finding has lead to a reworking of the inositol purification process.

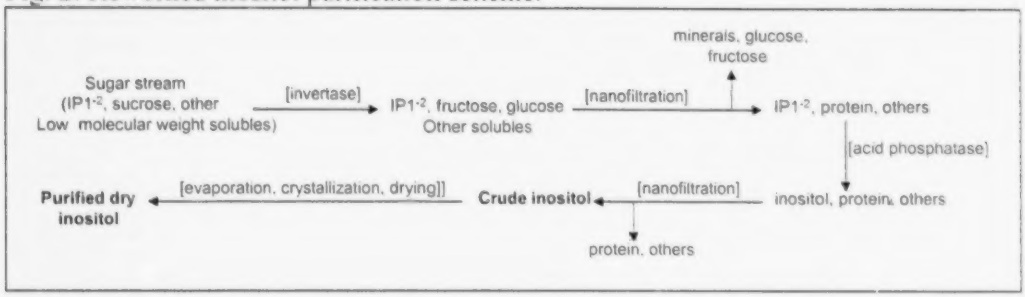
Phase 2: Reworking of the inositol purification process based on differential separation during nanofiltration.

Sucrose is the major soluble carbohydrate in the sugar stream produced by the MCN core process. Sucrose is a disaccharide composed of glucose and fructose. Invertase is a commercial enzyme preparation used for the inversion (hydrolysis) of sucrose to generate glucose and fructose. Pretreatment of the permeate from ultrafiltration with invertase should result in complete hydrolysis of sucrose to monosaccharides which should then be removed during the nanofiltration step. As such, by incorporation of an invertase pretreatment step, it should be possible to achieve complete separation of sugars from inositol phosphates during nanofiltration.

After sucrose hydrolysis, the sugar stream will be processed by nanofiltration with diafiltration to remove minerals and monosaccharides from the stream. The retentate from this step will be enriched in inositol phosphates and higher molecular weight constituents such as proteins.

Treatment of the retentate obtained from nanofiltration with an acid phosphatase should result in hydrolysis of inositol phosphates to inositol. As previously established inositol is poorly rejected by nanofiltration membranes. As such it should be possible to readily achieve a crude inositol preparation suitable for finishing by processing through the same nanofiltration membrane as used to separate monosaccharides from inositol phosphates in the first filtration.

Fig. 2. Reworked inositol purification scheme.



The reworked inositol purification scheme is shown in figure 2. Essentially, nanofiltration has replaced ion exchange as the basis for separation and production of the crude inositol stream. The system has the advantage of not requiring ion exchange resins, columns and regeneration chemicals. Further, issues of colored compounds irreversibly binding to the resin do not exist. As such the reworked process will provide both capital and operating cost advantages relative to the previous purification scheme.

Phase 3: Hydrolysis of sucrose by invertase. Conditions of temperature, pH, duration

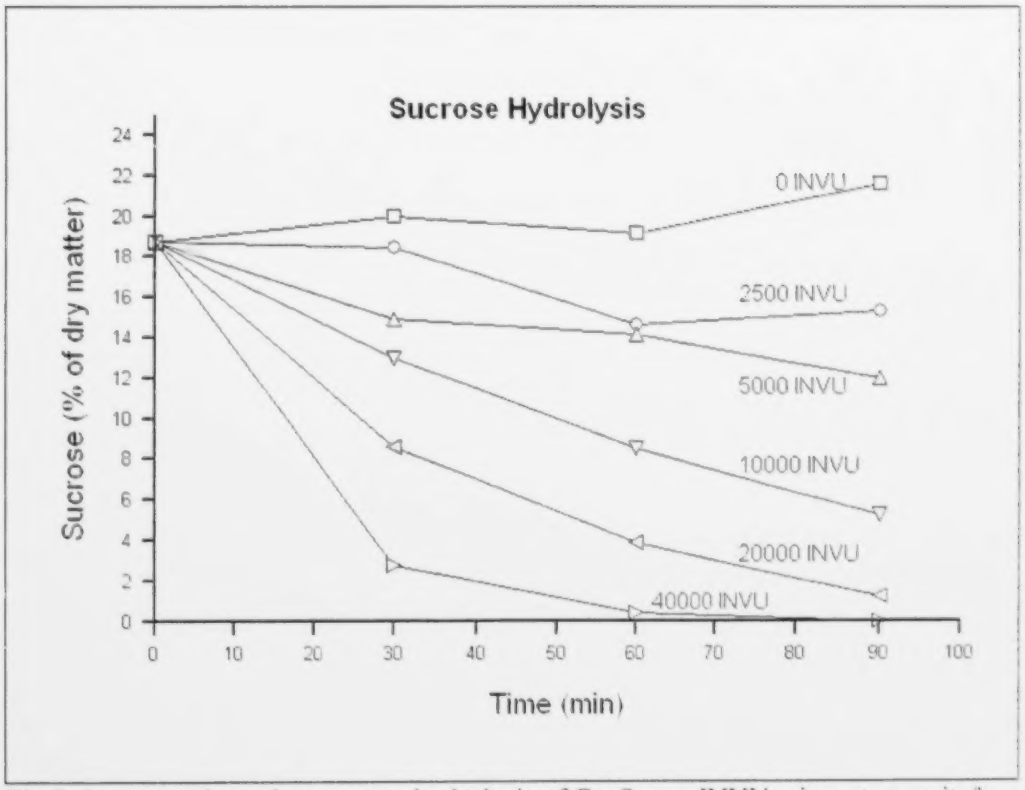


Fig 3. Invertase-dependent sucrose hydrolysis of CanSugar. INVU – invertase units/kg of dry matter.

and enzyme concentration for 100% hydrolysis of sucrose by invertase were established. Invertase has a broad pH optimum (4-6) and an optimum temperature of 50-55°C. Fig 3 summarizes the results of enzyme concentration and duration on the sucrose content of the CanSugar stream. Incubation for 90 min (50°C and pH 5.1) with 40000 units of invertase/kg of dry matter resulted in complete hydrolysis.

Ideal conditions of sucrose hydrolysis (temperature and pH) are similar to the conditions for phytate hydrolysis in the core process. As such it not necessary to introduce a distinct step for sucrose hydrolysis and this can be accomplished during the dephytinization procedure. The only adjustment to the process to achieve sucrose hydrolysis is to add invertase during this reaction step.

Phase 4: Processing of invertase-treated sugar stream by nanofiltration. Enzyme hydrolysis of sucrose yields glucose and fructose as products. Nanofiltration membranes are available in a several molecular weight cut-off. Dow supplies NF membranes with nominal molecular weight cut-offs of 200 (NF200) and 270 (NF270). Osmonics supplies NF membranes with cut-off of 180 (DK) and 300 (DL). These four nanofiltration membranes were screened for retention characteristics with invertase-treated CanSugar as the feed. Table 1 shows the glucose, fructose and inositol monophosphate and sucrose content of the feed and the permeate obtained from each membrane. As previously established, and shown in the sugar composition of the starting feed, treatment of CanSugar stream with invertase results in near complete hydrolysis of the sucrose and associated generation of glucose and fructose. The permeate from processing with each of the membranes was enriched in glucose and fructose which is consistent with passage of these sugars through the membrane. As a percentage of dry matter, inositol monophosphate levels in the permeate were much less than that of the starting feed indicating a high degree of retention by the membranes. The net result is a successful separation of the inositol precursors from glucose and fructose.

Table 1. Sugar and inositol monophosphate (IP1) content of permeate from processing invertase treated CanSugar through nanofiltration membranes

	% of Dry Matter				
	Fructose	Glucose	Inositol	IP1	Sucrose
Starting Feed	11.27	9.41	0.76	2.80	0.14
DK permeate	27.19	24.44	1.35	0.65	0.00
DL permeate	18.47	15.12	0.83	0.23	0.32
NF200 permeate	17.18	13.63	0.95	1.01	0.09
NF270 permeate	23.08	18.15	1.14	0.34	0.00

Phase 5: Hydrolysis of inositol phosphates to inositol. The next step in the purification scheme is to achieve enzymatic hydrolysis of inositol phosphates to inositol. Figure 4 shows the inositol contents in slurries of the sugar stream incubated with various concentrations of acid phosphatase under previously established conditions of pH (5.1) and temperature (50°C). The theoretical value for inositol generation from the precursor inositol phosphates is 3.4% of dry matter in the stream. After a 90 minute incubation with 40,000 acid phosphatase units/kg of dry matter inositol levels were 3.0% or 88% of

maximum. As such either a longer incubation period or a higher enzyme concentration should readily achieve complete hydrolysis and generation of myoinositol.

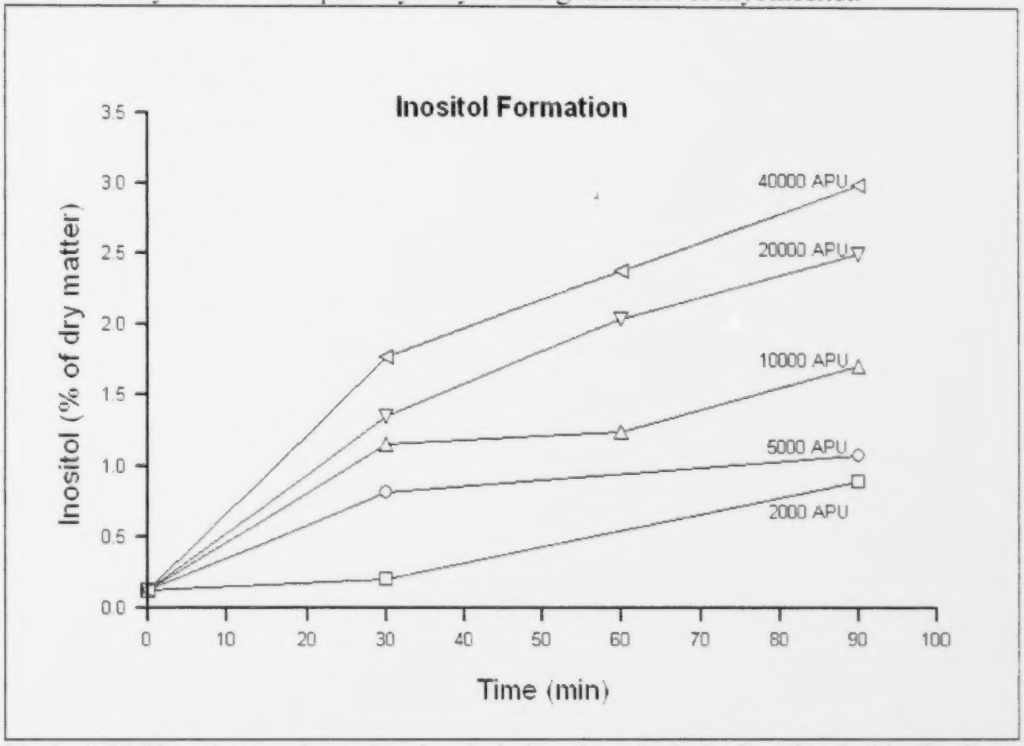


Fig 2. Acid Phosphatase-dependent inositol phosphate hydrolysis to inositol in CanSugar. APU – acid phosphatase units/kg of dry matter.

Phase 6: Generation of a crude inositol stream by nanofiltration. The final step in the crude purification scheme is to separate the inositol from the bulk of constituents in the acid phosphatase treated first nanofiltation pass retentate (see fig 1). Inositol has a moleculare weight of 180 daltons and should have similar retention characteristics to glucose when processed by nanofiltration. In theory inositol should pass freely through the membrane while the bulk of the remaining constituents of the retentate should be retained during the second retention step. In this experiment, CanSugar was treated with invertase to form glucose and fructose from sucrose. This sucrose-inverted CanSugar was then processed through a DL-nanofiltration membrane to remove monosaccharides and minerals and concentrate IP1 in the retentate. The first pass retentate was then treated with acid phosphatase to generate inositol. The theoretical level of inositol as a percentage of dry matter will be approximately double the level of inositol if CanSugar were to be directly treated with acid phosphatase. Table 2 shows the inositol concentration in the acid-phosphatase-treated feed, the second pass retentate and permeate. The inositol content of the feed material was 6.56% of dry matter which is a close approximation of the theoretical inositol content of this stream. Very little inositol was retained by the membrane. Inositol comprised 25% of the dry matter in the permeate. As such processing of acid phosphatase-treated first pass retentate by a second passage through a nanofiltration membrane successfully generated a crude inositol permeate.

Mass Balance and Further Processing of Crude Inositol. An assessment of mass and inositol flows through the purification scheme is consistent with total losses of inositol that approximated 50% of the theoretical yield. These losses consisted of non-extracted inositol equivalents of phytic acid, inositol phosphates losses in the liquid portion of other product streams and some losses in the filtration steps. This degree of product loss can be considered as acceptable and largely unavoidable given the number of steps in the process and the low concentration of inositol equivalents in the starting canola flake material.

Table 2. Inositol content of permeate and retentate after processing the acid phosphatase-treated first pass retentate through a DL nanofiltration membrane.

	Inositol		Non-Inositol Components	
	% of dry matter	% feed stream	% of dry matter	% feed stream
Feed	6.56	100	93.44	100
Permeate	25.39	75.1	74.61	15.49
Retentate	1.01	8.99	98.99	62.24
% Recovery		83.99		77.73

Final purification of the crude inositol stream will involve concentration by reverse osmosis, processing on activated carbon to remove residual colored and charged organic compounds, de-ashing (if needed), chromatographic separation of remaining sugars, evaporation, crystallization, and finally dewatering and drying of the pure product. A final purity of 80% is acceptable to end users of the product. These finishing steps are well established for finishing of glucose and fructose in the sweetener industry and these procedures represents a low risk portion of the overall process.